Genome Resource for the Huanglongbing Causal Agent ‘*Candidatus Liberibacter asiaticus*’ Strain AHCA17 from Citrus Root Tissue in California, USA

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Abstract

‘*Candidatus Liberibacter asiaticus*’ is the unculturable causative agent of citrus huanglongbing disease. Here, we report the first citrus root metagenome sequence containing the draft genome of ‘*Ca. L. asiaticus*’ strain AHCA17, obtained from a pummelo tree in California. The assembled genome was 1.2 Mbp and resulted in 37 contigs ($N_{50} = 158.7$ kbp) containing 1,057 predicted open reading frames and 45 RNA-coding genes. This draft genome will provide a valuable resource in further study of ‘*Ca. L. asiaticus*’ genome diversity and pathogen epidemiology.

‘*Candidatus Liberibacter asiaticus*’ is an unculturable bacteria and the causal agent of a serious citrus disease, huanglongbing (HLB) (Bové 2006; Jagoueix et al. 1994). HLB causes great economic losses annually in citrus production worldwide (Gottwald et al. 2007). Detection of ‘*Ca. L. asiaticus*’ is difficult due to uneven distribution in citrus tissues, low pathogen titer, and latency between infections and symptoms. A previous study showed that roots were one of the first tissues to show disease symptoms (Johnson et al. 2014). Currently, publicly available ‘*Ca. L. asiaticus*’ genomes from citrus samples were all obtained from foliar tissue (Cai et al. 2018; Dai et al. 2019; Wu et al. 2015; Zheng et al. 2014, 2018). Sequencing a ‘*Ca. L. asiaticus*’ genome from root tissue presents new challenges, considering the complex microbiota associated with this tissue (Zhang et al. 2017). Making additional ‘*Ca. L. asiaticus*’ genomes public provides a valuable supporting resource to understanding ‘*Ca. L. asiaticus*’ genome diversity, pathogen movement, and host interaction. Thus, here, we report the first draft whole-genome sequence of ‘*Ca. L. asiaticus*’ strain AHCA17, obtained directly from the root tissue of a pummelo tree (*Citrus maxima*) in Orange County, CA.

Genomic DNA of strain AHCA17 was extracted from root tissue using the DNeasy PowerPlant Pro kit (Qiagen, Valencia, CA, U.S.A.). The pathogen titer level was estimated using HLB *nrdB*-based real-time quantitative PCR, producing a threshold cycle value of 14.36 (Zheng et al. 2016), which signifies relatively high pathogen levels that are critical for successful sequencing. The 2 × 300-bp Illumina paired-end sequencing was performed on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, U.S.A.). The paired-end sequencing libraries were prepared using an Illumina Nextera DNA Flex library prep kit.

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Keywords

citrus, metagenomics, prokaryotes

Table 1. Comparison of bacterial diversity between a leaf sample and this root sample

Taxon ^c	Raw reads ^a		Percentage of previous taxon (%) ^b	
	Leaf	Root	Leaf	Root
Bacteria	1,081,337	1,644,680	4.19	10.28
Proteobacteria	448,570	705,316	41.48	42.88
α-Proteobacteria	58,495	363,619	13.04	51.55
Rhizobiales	36,633	274,884	62.63	75.60
Rhizobiaceae	23,783	95,018	64.92	34.57
Liberibacter	21,805	51,792	91.68	54.51

^a Total leaf sample (Cai et al. 2018) raw MiSeq reads numbered 25,830,723 and 15,992,407 for the root sample. Mapped using Kraken v1.1.1.

^b Percentage of reads composing immediately higher taxon (Bacteria indicates percentage of total reads).

^c Taxon lineage from Bacteria (superkingdom) to Liberibacter (genus).

In total, 3.19×10^7 reads with a mean of 269 bp/read were generated from MiSeq sequencing. Kraken (version 1.1.1) (Wood and Salzberg 2014) analysis revealed that 10.28% of the reads mapped to bacteria and not plant host (Table 1; mapped to complete bacterial, archeal, and viral Refseq genomes as of 18 July 2019). This is in contrast to a citrus leaf midrib sample we sequenced (5.16×10^7 reads with the same mean read length), which only had 4.19% of the reads map to bacteria (Cai et al. 2018). Interestingly, within bacteria, the Proteobacteria phylum was represented similarly between the root and leaf sample (41.48 and 42.88%, respectively). Within proteobacteria, there is an increase in diversity in the root sample compared with the leaf, exemplified by Liberibacter spp. representing 54.51% of the Rhizobiaceae family in the root sample and representing 91.68% in the leaf. The larger diversity of closely related organisms in the root led us to a map-to-pathogen, then assemble approach to analyzing the data.

Using the 'Ca. L. asiaticus' genome strain psy62 as a reference, 104,637 reads with 25,536,511 bases were aligned using Bowtie2 (version 2.3.0) covering 99.8% of the psy62 genome (Duan et al. 2009; Langmead and Salzberg 2012). Aligned reads were assembled using the SPAdes assembler (version 3.10.0) (Nurk et al. 2013) generating 37 contigs ≥ 500 bp, ranging from 500 to 266,672 bp ($N_{50} = 158.7$ kbp), with 24.56 average coverage. The contigs comprised a total length of 1,208,622 bp, with a G+C content of 36.39%, and aligned with 98.08% of the complete psy62 genome. Annotation was performed by prokka (version 1.13.3) (Seemann 2014). The genome included 1,057 predicted open reading frames and 45 RNA-coding genes.

This Whole-Genome Shotgun project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession VNFL00000000. The version described in this article is version VNFL01000000. Data has also been deposited in the Sequence Read Archive database (PRJNA552562).

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